REMARKS

Claims 1-15 are pending in the application upon entry of the amendments. The specification and claims 3, 4, 6, 8, 13 and 14 have been amended to better describe the invention. Favorable reconsideration in light of the amendments, the verified English translation of the priority document, and the remarks which follow is respectfully requested.

Claim for Priority

The Examiner has acknowledged the claim for foreign priority under 35 U.S.C. § 119. In support of the priority claim, enclosed herewith is a verified English translation of the priority document.

Amendment to the Specification

The specification has been amended to correct an obvious typographical error therein. In particular, on page 3, line 15, the number of Japanese Laid-Open Patent Publication No. 6-31627 has be changed to Japanese Laid-Open Patent Publication No. 6-316527. Entry is respectfully requested.

Allowable Subject Matter and Amendments

The Examiner's indication that the subject matter of claims 4, 5, 11, 12, 14, and 15 is allowable is noted with appreciation. Claims 4 and 14 have been amended into independent form. The term "an extract" in claims 3, 8 and 13 has been amended to "the polyalkoxyflavonoid extract" to more clearly define the extract. The word "preventing" in claims 6 and 8 has been deleted for clarification.

The §112 rejection

Claims 6-10 have been rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement with regard to using the invention. The Examiner contends that claims 6-10 do not meet the enablement requirement for preventing neurodegenerative diseases such as Alzheimer's disease. In accordance with the Examiner's suggestion,

the word "preventing" has been deleted from line 1 of claims 6 and 8. Withdrawal of the rejection is therefore respectfully requested.

The §102(e) rejection over Wenzel et al or Castillo et al

Claims 1, 2, 6, and 7 have been rejected under 35 U.S.C. § 102(e) over Wenzel et al. Claims 1 and 6 have been rejected under 35 U.S.C. § 102(e) over Castillo et al. The instant patent application has a U.S. filing date of August 9, 2001 and § 119 priority date of August 17, 2000. Wenzel et al has a U.S. filing date of February 14, 2001 and a publication date of November 29, 2001. The date at which Wenzel et al is prior art is it's U.S. filing date of February 14, 2001, assuming it meets § 112, first paragraph requirements. Since the § 119 priority date of August 17, 2000 of the instant patent application is before the filing date of February 14, 2001 of Wenzel et al, Wenzel et al is not prior art against the instant patent application.

Castillo et al has a U.S. filing date of December 26, 2000 and a publication date of November 29, 2001. The date at which Castillo et al is prior art is it's U.S. filing date of December 26, 2000, assuming it meets § 112, first paragraph requirements. Since the § 119 priority date of August 17, 2000 of the instant patent application is before Castillo et al's filing date of December 26, 2000, Castillo et al is not prior art against the instant patent application.

Further in support of the patentability of the instant application, a verified English translation of the priority document is enclosed herewith. The enclosed verified English translation of the priority document and the § 119 priority date of the instant patent application render the § 102(e) rejections moot.

The §102(e) rejection over Obukowicz et al

Claims 3 and 8 have been rejected under 35 U.S.C. § 102(e) over Obukowicz et al. Obukowicz et al has a U.S. filing date of December 15, 2000, which is later than the §119 priority date of the instant application (August 17, 2000). Obukowicz et al is a CIP application of an application having a U.S. filing date of March 19, 1999. The CIP

subject matter of Obukowicz et al is not prior art against the instant application for the same reasons that Wenzel et al and Castillo et al are not prior art.

The original application of Obukowicz et al filed on March 19, 1999 merely discloses that the extract from Atractylodes lancea has an inhibitory effect on COX-2 activity associated with the inflammation process. That is, the original application of Obukowicz et al filed on March 19, 1999 describes that an organic extract of an edible plant inhibits COX-2, and COX-2 mediates inflammation or inflammation-associated disorders.

To establish anticipation, each and every claim feature must be disclosed in a single cited art document. Claims 3 and 8 require extending neutites by administering, in part, a polyalkoxyflavonoid extract. The original application of Obukowicz et al fails to disclose, teach, or suggest that polyalkoxyflavonoids have an effect on extending neurites. In particular, the original application of Obukowicz et al fails to disclose, teach, or suggest or even mention polyalkoxyflavonoids as well as their effect on extending neurites. Since Obukowicz et al does not disclose each and every element of claims 3 and 8, Obukowicz et al cannot anticipate claims 3 and 8. Withdrawal of the rejection is therefore respectfully requested.

The §102(b) rejection over JP8-81382

Claims 3 and 8 have been rejected under 35 U.S.C. § 102(b) over JP 8-81,382. JP '382 relates to orally administering the extract of a citrus plant. The Examiner contends that JP '382 inherently anticipates claims 3 and 8.

JP '382 merely describes that lipolysis of neutral fat accumulated in adipose tissue can be accelerated by administrating a plant extract belonging to the citrus family. The Examiner contends that JP '382 discloses administrating the same extract as claims 3 and 8. However, JP '382 fails to disclose, teach, or suggest the effective ingredient in the extract on lipopysis, and further that the extract has an effect on extending neurite. The present inventors are the first to discover that a polyalkoxyflavonoid extract (or the extract containing polyalkoxyflavonoid) has an effect

on extending neurite. Furthermore, JP '382 fails to suggest the relationship between a lypolysis-accelerating effect and a neurite-extending effect.

In sum, JP '382 fails to disclose, teach, or suggest that polyalkoxyflavonoids have an effect on extending neurites. Since JP '382 does not disclose each and every element of claims 3 and 8, JP '382 cannot anticipate claims 3 and 8. Withdrawal of the rejection is therefore respectfully requested.

The §103(a) rejection over JP8-81382 and Lanzendorfer et al

Claim 13 has been rejected under 35 U.S.C. § 103(a) over JP '382 and Lanzendorfer et al. Lanzendorfer et al is cited for the proposition of describing flavonoids. Lanzendorfer et al discloses that flavonoids or flavonoids in combination with cinnamic acid derivatives can treat "stinging" sensations on the skin due to their antioxidative effects. In other words, Lanzendorder et al fails to teach or suggest that citrus extracts containing flavonoids have an effect on extending neurites. As described above, in JP '382, there is no description or suggestion of an effect by a polyalkoxyflavonoid extract on extending neurites.

Since both JP '382 and Lanzendorfer et al fail to teach or suggest that a polyalkoxyflavonoid extract containing flavonoids has an effect on extending neurites, one skilled in the art would not have reached the method of claim 13 even if these disclosures are combined. Moreover, one skilled in the art would not have combined the flavonoids of Lanzendorfer et al in the composition of JP '832 because JP '832 is concerned with lipolysis of neutral fat whereas Lanzendorfer et al is not concerned with the lipolysis of neutral fat.

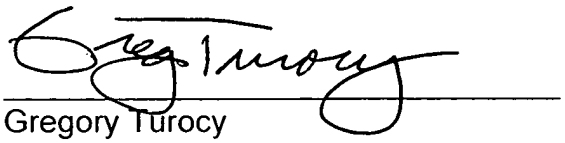
For either or both these reasons, the subject matter of claim 13 is not rendered obvious over JP '382 and Lanzendorfer et al.

Should the Examiner believe that a telephone interview would be helpful to expedite favorable prosecution, the Examiner is invited to contact Applicants' undersigned attorney at the telephone number listed below.

In the event any fees are due in connection with the filing of this document, the Commissioner is authorized to charge those fees to our Deposit Account No. 50-1063.

Respectfully submitted,

AMIN & TUROCY, LLP



Gregory Turocy
Reg. No. 36,952

24th Floor, National City Center
1900 East 9th Street
Cleveland, Ohio 44114
(216) 696-8730
Fax (216) 696-8731

VERIFICATION OF TRANSLATION

I, Yoko Hamaguchi of 5-5-4-404, Yokozutsumi, Tsurumi-ku, Osaka-shi, Osaka 538-0052 Japan

declare as follows:

That I am well acquainted with both the English and Japanese languages, and

That the attached document is true and correct translation made by me to the best of my knowledge and belief of:

Japanese Patent Application No. 2000-248021

Date: March 12, 2003

Yoko Hamaguchi

(Signature of Translator)

[Name of Document] PATENT APPLICATION

[Case Number] 0054JP01

[Addressee] Commissioner, Patent Office

[International Patent Classification] A61K 31/35

[Inventor]

[Address] c/o Research & Development Center of Nagase & Company, Ltd., 2-3, Murotani 2-chome, Nishi-ku, Kobe-shi, Hyogo

[Name] Hisatomi ITO

[Inventor]

[Address] c/o Research & Development Center of Nagase & Company, Ltd., 2-3, Murotani 2-chome, Nishi-ku, Kobe-shi, Hyogo

[Name] Shinya TAMURA

[Inventor]

[Address] c/o Research & Development Center of Nagase & Company, Ltd., 2-3, Murotani 2-chome, Nishi-ku, Kobe-shi, Hyogo

[Name] Toshitsugu MIYAZAKI

[Patent Applicant]

[Identification Number] 000214272

[Name] Nagase & Company, Ltd.

[Representative] Hiroshi Nagase

[Official Fee]

[Advance Payment Note Number] 094283

[Amount of Payment] 21,000 yen

[List of File Documents]

[Item] Patent Specification 1

[Item] Abstract 1

[Proof] yes

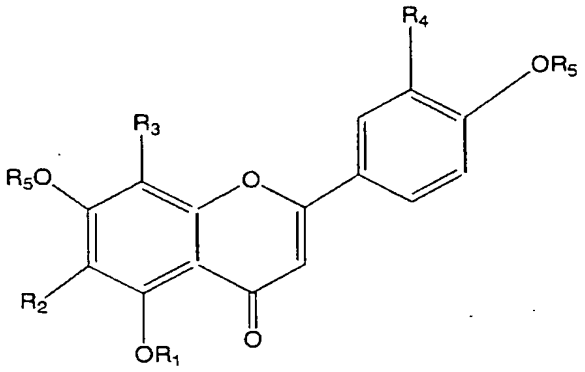
[Name of Document] SPECIFICATION

[Title of the Invention] Neurite extending agent

[Claims]

1. A neurite extending agent comprising a polyalkoxyflavonoid represented by Formula (1):

[Compound 1]



(1)

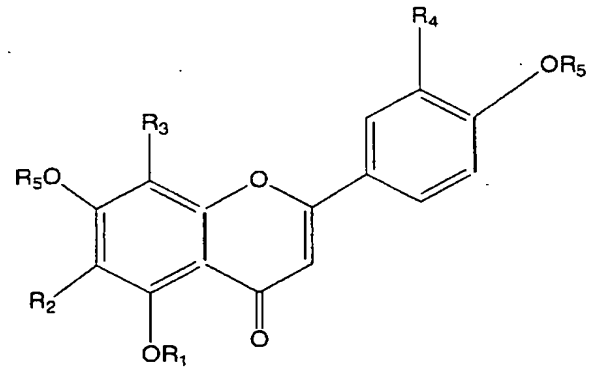
wherein R_1 is H or a lower alkyl group of C_1 to C_6; R_2, R_3 and R_4 are each independently H or an alkoxy group of C_1 to C_6; and R_5 is a lower alkyl group of C_1 to C_6.

2. The neurite extending agent of claim 1, wherein the polyalkoxyflavonoid represented by Formula (1) is nobiletin or tangeretin.

3. A neurite extending agent comprising an extract from a plant belonging to the citrus family.

4. The neurite extending agent of claim 3, wherein the extract from a plant belonging to the citrus family comprises a polyalkoxyflavonoid represented by Formula (1):

[Compound 2]



(1)

wherein R_1 is H or a lower alkyl group of C_1 to C_6; R_2, R_3 and R_4 are each independently H or an alkoxy group of C_1 to C_6; and R_5 is a lower alkyl group of C_1 to C_6.

5. The neurite extending agent of claim 4, wherein the polyalkoxyflavonoid represented by Formula (1) is nobiletin or tangeretin.

6. The neurite extending agent of any one of claims 1 to 5, wherein the agent is manufactured in a form of a quasi-drug or a food product.

[Detailed Description of the Invention]

[0001]

[Field of the Invention]

The present invention relates to a novel neurite extending agent having neurite extending effect. More specifically, the present invention relates to a neurite extending agent useful for preventing and/or improving or treating neurodegeneration diseases such as Alzheimer's dementia and cerebral ischemia by accelerating neurite extension.

[0002]

[Prior Art]

With the shift to the aging society, the incidence of senile dementia has been increasing and this has become a serious social problem. Many diseases are known to cause senile dementia. Senile dementia is roughly classified into three types: dementia due to organic disorders of the brain; dementia associated with diseases of organs other than brain; and dementia due to physical diseases caused by stress. In particular, senile dementia is caused mostly by organic disorders of the brain, and the dementia of this type is further classified into two types, cerebrovascular dementia and Alzheimer's dementia, depending on its cause.

[0003]

It is known today that cerebral vasodilators have some effect on cerebrovascular dementia. However, concerning Alzheimer's dementia, the cause of this disease is still unknown and there is no report on treatment methods or pharmacotherapy suitable to prevent its pathopoiesis as well as

its advance. Therefore, there is a need to develop medicines that are effective with respect to the dementia caused by organic disorders of the brain, especially Alzheimer's dementia.

[0004]

In recent years, neurotrophical factors secreted from neurocytes such as nerve growth factors (NGF) have been found to exhibit excellent effects on neurodegeneration diseases and have attracted public attention. An NGF is a factor that is important and necessary for nervous tissue to grow and maintain its function. An NGF is indispensable for the maturation, differentiation and viability of sensory nerves and sympathetic nerves in the peripheral nerves, and of magnocellular cholinergic neuron in the central nerves. An NGF also acts to prevent degeneration of neurocytes when the brain is damaged. In this regard, raising the NGF level in a living body seems to be effective as a treatment method for disorders of central function, such as Alzheimer's dementia and cerebrovascular dementia, spinal cord injuries, peripheral nerve injuries, diabetic neuropathy and disorder of peripheral function such as amyotrophic lateral sclerosis.

[0005]

However, an NGF is a protein having a molecular weight of 13000 in the form of monomer and 26000 in the form of dimer, so that it cannot pass through the blood-brain barrier. Therefore, in order to treat disorders of central function, NGFs are required to be administrated intraventricularly. Moreover, it is difficult to prepare NGFs in large quantities. In these respects, there are many problems about the use of NGF itself. As a result, it is very difficult to use NGF itself clinically.

[0006]

Y. Furukawa et al. disclose the use of catecholamines (epinephrine, norepinephrines) as an NGF synthesis accelerator (FEBS Lett., 208, 258 (1986)). Further, it is disclosed that theanine (Japanese Laid-Open Patent Publication (Tokkai) No. 7-173059), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Japanese Laid-Open Patent Publication (Tokkai) No. 8-143454) serve as an NGF synthesis accelerator.

[0007]

However, since epinephrine and norepinephrine are hormones, there is the problem that the quantitative balance of hormones in a living body may be lost if such a substance is administrated. It is another drawback that the above-described NGF synthesis accelerator may exhaust brain cells that are already in abnormal conditions because the NGF synthesis accelerator forcibly releases NGFs.

[0008]

Therefore, in order to prevent and/or improve or treat senile dementia, low molecular weight substances that exhibit NGF-like activity appear to be effective.

[0009]

On the other hand, with respect to polyalkoxyflavonoid, Japanese Laid-Open Patent Publication (Tokkai) No. 2000-80035 only describes that it has matrix metalloprotease inhibitory effect. Japanese Laid-Open Patent Publication (Tokkai) No.6-31627 has reported that alcoholic extracts of ginseng have an activating effect on neurocytes, but the substance that has the activating effect has not been specified.

[0010]

[Problems to be Solved by the Invention]

The present invention is to solve the foregoing problems, and it is an object of the present invention to provide a novel neurite extending agent having neurite extending effect without any side effects.

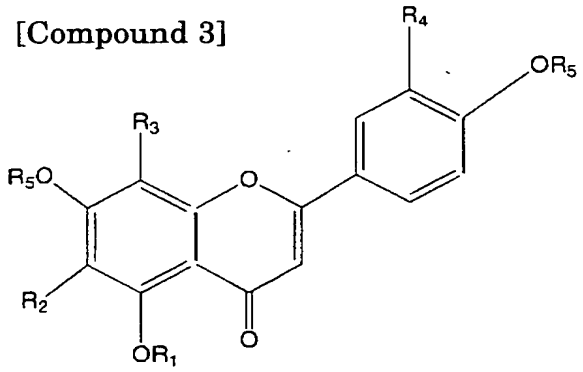
[0011]

[Means for Solving the Problems]

The present invention provides a neurite extending agent comprising a polyalkoxyflavonoid represented by Formula (1):

[0012]

[Compound 3]



(1)

[0013]

wherein R_1 is H or a lower alkyl group of C_1 to C_6; R_2, R_3 and R_4 are each independently H or an alkoxy group of C_1 to C_6; and R_5 is a lower alkyl group of C_1 to C_6. By these agents, the above-mentioned problems can be solved.

[0014]

In a preferable embodiment, the polyalkoxyflavonoid represented by Formula (1) is nobiletin or tangeretin.

[0015]

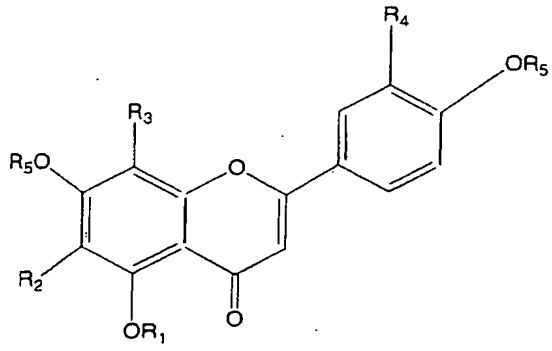
The present invention also provides a neurite extending agent comprising an extract from a plant belonging to the citrus family.

[0016]

In a preferable embodiment, the extract from a plant belonging to the citrus family comprises a polyalkoxyflavonoid represented by Formula (1):

[0017]

[Compound 4]



(1)

[0018]

wherein R_1 is H or a lower alkyl group of C_1 to C_6; R_2, R_3 and R_4 are each

independently H or an alkoxy group of C_1 to C_6; and R_5 is a lower alkyl group of C_1 to C_6.

[0019]

In a further preferable embodiment, the polyalkoxyflavonoid represented by Formula (1) is nobiletin or tangeretin.

[0020]

In a preferable embodiment, the agent is manufactured in a form of a quasi-drug or a food product.

[0021]

[Modes for Carrying out the Invention]

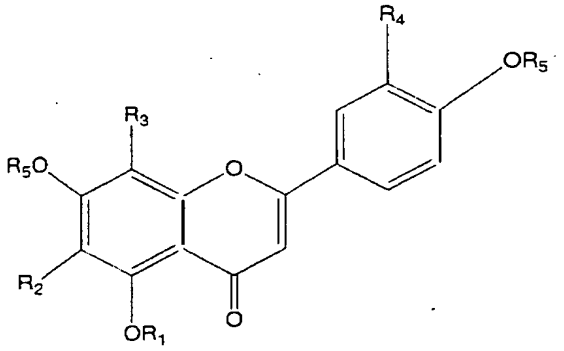
It is known that PC12 cells derived from adrenal medulla pheochromocytoma of rats extend neurites in response to NGFs. The inventors of the present invention examined various substances having NGF-like activities, using the evaluation system. As a result, the inventors of the present invention discovered that a polyalkoxyflavonoid having a specific chemical structure exhibits an excellent neurite extending effect.

[0022]

Therefore, a neurite extending agent of the present invention contains a polyalkoxyflavonoid as represented by Formula (1):

[0023]

[Compound 5]



(1)

[0024]

wherein R_1 is H or a lower alkyl group of C_1 to C_6; R_2, R_3 and R_4 are each independently H or an alkoxy group of C_1 to C_6; and R_5 is a lower alkyl group of C_1 to C_6. R_1 is preferably H or a lower alkyl group of C_1 to C_3. Preferably,

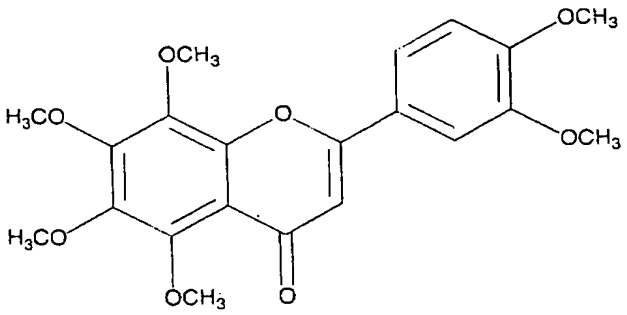
R_2, R_3 and R_4 are each independently H or an alkoxy group of C_1 to C_3. R_5 is preferably a lower alkyl group of C1 to C3.

[0025]

As examples of the polyalkoxyflavonoids represented by Formula (1), nobiletin represented by Formula (2) and tangeretin represented by Formula (3) are preferable because of the stability of these substances and the presence in a large amount in plants belonging to the citrus family:

[0026]

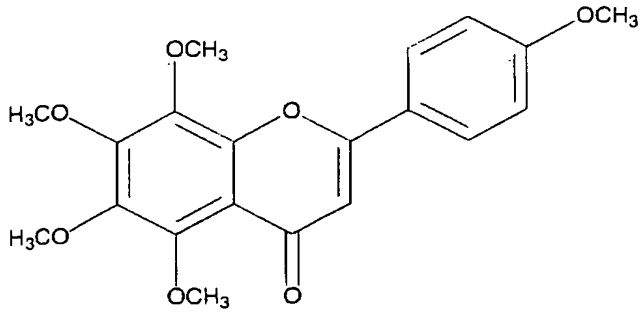
[Compound 6]



(2)

[0027]

[Compound 7]



(3)

[0028]

Nobiletin and tangeretin are contained in a large amount in plants belonging to the citrus family. Tangeretin is commercially available.

[0029]

In the present invention, the content of polyalkoxyflavonoid represented by Formula (1) is 0.00001% by weight through 50% by weight, preferably 0.0001% by weight through 30% by weight as an effective

ingredient in 100% by weight of the neurite extending agent. If the polyalkoxyflavonoid content is less than 0.00001% by weight, the neurite extending effect may not reach the desired level. On the other hand, if the content exceeds 50% by weight, better effects may not be expected. In the present invention, polyalkoxyflavonoids as described above can also be used in combination of two or more.

[0030]

The polyalkoxyflavonoid represented by Formula (1) that is used in the present invention can be synthesized chemically, using methods well known to those skilled in the art. Alternatively, the polyalkoxyflavonoid can also be easily extracted from plants belonging to the citrus family as described later. In particular, a methoxyflavonoid may be extracted and isolated from plants by the method as described by Jie Chem et al.(J. Agric. Food. Chem., 45, 364-368 (1997)).

[0031]

The composition of the present invention may also contain an extract from a plant belonging to the citrus family.

[0032]

Examples of the plants belonging to the citrus family that are used for extraction include Hirami-lemon (Citrus depressa), Unshiu-mikan (Citrus unshiu), Ohbeni-mikan (Citrus tangerina), Kobeni-mikan (Citrus erythrosa), Daidai (Citrus aurantium), Natsu-mikan (Citrus natsudaidai), Zabon (Citrus grandis), Yuzu (Citrus Junos), Ponkan (Citrus rericulata), Lemon (Citrus lemon), Karatachi (Citrus trifoliata) and Marubushukan (Citrus medica L.), all belonging to the citrus genus. Among these, Hirami-lemon, Unshiu-mikan and Daidai are preferable. In the present invention, the extracts from the above plants belonging to the citrus family may be used in combination of two or more.

[0033]

The extracts from the plants belonging to the citrus family used in the present invention may be obtained by extraction either from fresh plants or dried plants after collection. As for the parts to be used, fruits and peel of

mature or immature plants, seeds, leaves, leafstalks, branches, roots and flowers of plants can be used. In particular, fruits and peel are preferable.

[0034]

For example, extracts from the plants belonging to the citrus family can be obtained in the following manner.

[0035]

First, a specified part of a plant belonging to the citrus family is immersed in an extractant. The amount of the extractant can be any amount as long as the plant is immersed in it, but amounts of twice to 100 times the weight of the plant belonging to the citrus family are preferable. There is no particular limitation regarding the extractant to be used. Examples of possible extractants to be used include lower alcohols such as methanol, ethanol, n-propanol, isopropanol and t-butanol; ketones such as acetone; esters such as ethylester acetate; ethers; organic solvents such as chloroform and dichloromethane; and water. These extractants can be used alone or in combination. In the present invention, methanol, ethanol, ethyl acetate, or combinations of these extractants with water are preferable. Considering the safety in a living body, ethanol or a mixed solvent of water and ethanol is more preferable because of their low toxicity. There is no particular limitation regarding the other conditions such as extracting temperatures, which can be set as appropriate by those skilled in the art.

[0036]

The extract from a plant belonging to the citrus family obtained in this manner contains polyalkoxyflavonoid represented by Formula (1), preferably nobiletin represented by Formula (2) and tangeretin represented by Formula (3). These can be isolated and obtained from the extract of a plant belonging to the citrus family by column chromatography. Thus obtained substance can be identified as nobiletin or tangeretin by well-known means such as ^{1}H-NMR and ^{13}C-NMR.

[0037]

Regarding tengeretin, it can be commercially available.

[0038]

When nobiletin or tangeretin which is an typical example of polyalkoxyflavonoid represented by Formula (1) is used as a substitute for the extract from a plant belonging to the citrus family containing the compound, the content of the extract is the same as in polyalkoxyflavonoid as described above. Preferably, in 100% by weight of the neurite extending agent of the present invention, the extract content is preferably 0.00001% by weight through 30% by weight, more preferably 0.0001% by weight through 15% by weight. If the content of extract, nobiletin, or tangeretin is less than 0.00001% by weight, the effect cannot appear sufficiently.

[0039]

The neurite extending agent of the present invention can be contained in medicaments, quasi-drugs, or food products. The neurite extending agent of the present invention can be also used either for oral administration or for parenteral administration.

[0040]

Examples of the forms of the medicaments are tablets, capsules, granules, and syrup. These medicaments can be produced by using pharmaceutically acceptable carriers that are commonly used for production of medicaments.

[0041]

Examples of the pharmaceutically acceptable carriers are lactose, dextrin, sucrose, mannitol, cornstarch, sorbitol, crystalline cellulose and polyvinylpyrrolidone. These can be used alone or in combination as appropriate. The medicament can be produced by a method suitable for the form of each pharmaceutical under Japanese Pharmacopeia. Furthermore, additives such as seasoning agents, coloring matters and sweetening agents can also be used, if necessary. The content of these additives can be selected as appropriate by those skilled in the art.

[0042]

Examples of the forms of the quasi-drugs are tablets, capsules, granules, jellies and drinkable preparations. These quasi-drugs can be produced by using pharmaceutically acceptable carriers that are commonly

used for production of quasi-drugs. Furthermore, the quasi-drugs can contain other active ingredients such as vitamins. Additives such as sweetening agents, seasoning agents, coloring matters and antioxidants can also be used alone or in combination as appropriate. The quasi-drug can be produced by a method well known to those skilled in the art.

[0043]

Examples of the forms of the food products are noodles, pasta, granules, tablets, jelly and liquid (drink). These food products can be produced using various kinds of food ingredients as appropriate. Specific examples of food ingredients include rice, wheat, corn, potatoes, sweet potatoes, soybean meal, seaweed powder, starch syrup, lactose, glucose, fructose, sucrose and mannitol. These can be used alone or in combination as appropriate. By using water or the like, if necessary, the food products can be made into the desired form. Furthermore, seasoning agents, coloring matters, sweetening agents, edible oil, vitamins and the like can be added as appropriate.

[0044]

[Examples]

Hereinafter, examples of the present invention will be described. The present invention is not limited by these examples.

[0045]

<Example 1>

First, 500g of dried immature peel of Seihi (immature peel of Citrus unshiu) were subjected to extraction with 90(v/v) % ethanol by commonly used manner. An extract was filtered and concentrated under reduced pressure, and 28.2g of residue were obtained. The residue was separated with ethyl acetate-water, and then an ethyl acetate layer was concentrated under reduced pressure to obtain 11.6g of an extract of Seihi.

[0046]

The extract of immature peel of Seihi was subjected to silica gel chromatography (eluent; ethyl acetate : n-hexane (1:1)), and fractionation was performed by HPLC (eluent: A. 2% acetic acid aqueous solution, B.

acetonitrile; A:B=85%:15% for 5 minutes, then gradient from A:B=85%:15% to A:B=40%:60% for 30 minutes; Flow rate: 10 ml/min.; Detection: UV 340nm). The fractions obtained at retention times of 28.5 minutes and 30.5 minutes were concentrated, dried, and crystallized with diethyl ether to obtain crystalline substances (1) and (2), respectively. The melting point of the substance (1) measured by commonly used manner was 137 C to 138 C. The results of ^{13}C-NMR and ^{1}H-NMR of the substance (1) are shown in Table 1.

[0047]

[Table 1]

NMR spectrum of the substance (1)	
^{13}C-NMR, δ (ppm)	^{1}H-NMR, δ (ppm)
55.6(OMe), 55.7(OMe), 61.4(OMe), 61.5(OMe), 61.8(OMe), 61.9(OMe), 106.3(CH), 108.9(CH), 111.8(CH), 114.3(C), 119.3(CH), 123.1(C), 137.7(C), 143.5(C), 147.5(C), 149.0(C), 150.9(C), 151.7(C), 160.7(C), 175.8(C=O)	3.77(s, 3H), 3.83(s, 3H), 3.84(s, 3H), 3.87(s, 3H), 3.96(s, 3H), 4.01(s, 3H), 6.85(s, 1H), 7.15(d, J=8.6Hz, 1H), 7.53(d, J-2.1Hz, 1H), 7.64(dd, J=2.1, 8.6Hz, 1H)

[0048]

On the other hand, the melting point of the substance (2) measured by commonly used manner was 156 Cto 157 C. The results of ^{13}C-NMR and ^{1}H-NMR of the substance B are shown in Table 2.

[0049]

[Table 2]

NMR spectrum of the substance (2)	
^{13}C-NMR, δ (ppm)	^{1}H-NMR, δ (ppm)
55.5(OMe), 61.6(OMe), 61.8(OMe), 62.0(OMe), 62.2(OMe), 106.7(CH), 114.5(CHx2), 114.9(C), 123.8(CHx2), 138.1(C), 144.1(C), 147.7(C), 148.4(C), 151.3(C), 161.2(C), 162.3(C), 176.8(C=O)	3.88(s, 3H), 3.94(s, 3H), 4.02(s, 3H), 4.09(s, 3H), 6.59(s, 1H), 7.01(d, J=8.8Hz, 2H), 7.86(d, J=8.8Hz, 2H)

[0050]

Comparing the results of the measurement of the substances (1) and (2) with the values described in J. Agric. Food. Chem., 45, 364-368, (1997),

the obtained substance (1) was identified as nobiletin and the substance (2) as tangeretin.

[0051]

After the identification, the extract of Seihi was used without any further treatment as a test material A (neurite extending agent).

[0052]

Next, PC 12 cells derived from adrenal medulla pheochromocytoma of rats were seeded in a serum-free DMEM/F12 medium containing 5μg/ml of transferrin, 5μg/ml of insulin and 20 nM of progesterone (GIBCO Corp., hereinafter, referred to as "DMEM-TIP medium") at 2.0×10^4 cells/well (flat-bottomed 24 well collagen coated plate, manufactured by IWAKI). Then, the cells were cultured overnight at 37°C under 5% CO_2.

[0053]

Thereafter, the PC12 cells were removed from the medium, transferred to the DEMEM-TIP medium containing 10μg/ml of the above test material A and further cultured for 3 days.

[0054]

After culturing for 3 days, for each well of the plate, microscopic observation was conducted with respect to the cells at 200 times magnification. The percentage of the cells with extended neurites (cells that have neurites longer than their diameter) to the total of more than 200 cells was calculated. The results are shown in Table 3.

[0055]

<Example 2>

The percentage of the cells with extended neurites was calculated in the same manner as in Example 1 except that PC12 cells were transferred to the DEMEM-TIP medium containing 50μg/ml of the test material A. The results are shown in Table 3.

[0056]

<Example 3>

First, 500g of dried peel of Hirami-lemon (Citrus depressa) were subjected to extraction with 90 (v/v) % ethanol by commonly used manner.

An extract was filtered and concentrated under reduced pressure, and 19.6g of residue was obtained. Then, the residue was separated with ethyl acetate-water, and an ethyl acetate layer was concentrated under reduced pressure to obtain 8.5g of an extract of Hirami-lemon.

[0057]

Analyzing the obtained extract of Hirami-lemon by HPLC (eluent: A. 2% acetic acid aqueous solution, B. acetonitrile; A:B=85%:15% for 5 minutes, then gradient from A:B=85%:15% to A:B=40%:60% for 30 minutes; Flow rate: 10 ml/min.; Detection: UV 340nm), peaks corresponding to nobiletin (retention time: 28.2 minutes) and tangeretin (retention time: 30.5 minutes) were found.

[0058]

The percentage of the cells with extended neurites was calculated in the same manner as in Example 1 except that the extract from Hirami-lemon was used without any further treatment as a test material B instead of the test material A, and that PC12 cells were transferred to the DEMEM-TIP medium containing 10μg/ml of the test material B. The results are shown in Table 3.

[0059]

<Example 4>

The percentage of the cells with extended neurites was calculated in the same manner as in Example 3 except that PC12 cells were transferred to the DEMEM-TIP medium containing 50μg/ml of the test material B. The results are shown in Table 3.

[0060]

<Example 5>

First, 500g of dried peel of Touhi (peel of Citrus aurantium) were subjected tor extraction with 90 (v/v) % ethanol by commonly used manner. An extract was filtered and concentrated under reduced pressure, and 18.5g of residue were obtained. Then, the residue was separated with ethyl acetate-water, and then an ethyl acetate layer was concentrated under reduced pressure to obtain 7.2g of an extract of Touhi.

[0061]

Analyzing the obtained extract of Touhi by HPLC (eluent: A. 2% acetic acid aqueous solution, B. acetonitrile; A:B=85%:15% for 5 minutes, then gradient from A:B=85%:15% to A:B=40%:60% for 30 minutes; Flow rate: 10 ml/min.; Detection: UV 340nm), peaks corresponding to nobiletin (retention time: 28.2 minutes) and tangeretin (retention time: 30.5 minutes) were found.

[0062]

The percentage of the cells with extended neurites was calculated in the same manner as in Example 1 except that the extract from Touhi was used without any further treatment as a test material C instead of the test material A, and that PC12 cells were transferred to the DEMEM-TIP medium containing 10μg/ml of the test material C. The results are shown in Table 3.

[0063]

<Example 6>

The percentage of the cells with extended neurites was calculated in the same manner as in Example 5 except that PC12 cells were transferred to the DEMEM-TIP medium containing 50μg/ml of the test material C. The results are shown in Table 3.

[0064]

<Example 7>

The percentage of the cells with extended neurites was calculated in the same manner as in Example 1 except that nobiletin obtained in Example 1 was used without any further treatment as a test material D instead of the test material A and that PC12 cells were transferred to the DEMEM-TIP medium containing 10μM of the test material D. The results are shown in Table 3.

[0065]

<Example 8>

The percentage of the cells with extended neurites was calculated in the same manner as in Example 1 except for using nobiletin obtained in

Example 1 was used without any further treatment as a test material D instead of the test material A, and that PC12 cells were transferred to the DEMEM-TIP medium containing 50μM of the test material D. The results are shown in Table 3.

[0066]

<Example 9>

The percentage of the cells with extended neurites was calculated in the same manner as in Example 1 except that nobiletin obtained in Example 1 was used without any further treatment as a test material D instead of the test material A, and that PC12 cells were transferred to the DEMEM-TIP medium containing 100μM of the test material D. The results are shown in Table 3.

[0067]

<Example 10>

The percentage of the cells with extended neurites was calculated in the same manner as in Example 1 except that tangeretin obtained in Example 1 was used without any further treatment as a test material E instead of the test material A, and that PC12 cells were transferred to the DEMEM-TIP medium containing 10μM of the test material E. The results are shown in Table 3.

[0068]

<Example 11>

The percentage of the cells with extended neurites was calculated in the same manner as in Example 1 except tangeretin obtained in Example 1 was used without any further treatment as a test material E instead of the test material A, and that PC12 cells were transferred to the DEMEM-TIP medium containing 100μM of the test material E. The results are shown in Table 3.

[0069]

<Comparative Example 1>

The percentage of the cells with extended neurites was calculated in the same as in Example 1 except that PC12 cells were transferred to the

DEMEM-TIP medium that did not contain the test material A of Example 1. The percentage in this case was used as a control. The results are shown in Table 3.

[0070]

<Comparative Example 2>

The percentage of the cells with extended neurites was calculated in the same manner as in Example 1 except that dibutyl cyclic AMP that has been reported to have neurite extending effect (Nerochem. Int. 33, 503, (1999)) was used without any further treatment as a test material F instead of the test material A, and that PC12 cells were transferred to the DEMEM-TIP medium containing 100μM of the test material F. The results are shown in Table 3.

[0071]

<Comparative Example 3>

The percentage of the cells with extended neurites was calculated in the same manner as in Example 1 except that isobutylmethylxanthine that has been reported to have neurite extending effect (J. Neurobiol. 19 (8), 681, (1988)) was used without any further treatment as a test material G instead of the test material A, and that PC12 cells were transferred to the DEMEM-TIP medium containing 100μM of the test material G. The results are shown in Table 3.

[0072]

[Table 3]

	Active ingredient in the test material	Concentration of a composition for extending neurites of the present invention in the medium	Ratio of the cells with extended neurites (%)	Relative value of the ratio of the cells with extended neurites to control (Com.Ex.1)[1)]
Ex.1	extract of immature peel of Citrus unshiu	10μg/ml	10.2	2.9
Ex.2	extract of immature peel of Citrus unshiu	50μg/ml	18.6	5.3

Ex.3	extract of Citrus depressa	10μg/ml	7.5	2.1
Ex.4	extract of Citrus depressa	50μg/ml	16.8	4.8
Ex.5	extract of Citrus aurantium	10μg/ml	7.1	2.0
Ex.6	extract of Citrus aurantium	50μg/ml	15.9	4.5
Ex.7	nobiletin	10μM	8.7	2.5
Ex.8	nobiletin	50μM	16.6	4.7
Ex.9	nobiletin	100μM	42.8	12.2
Ex.10	tangeretin	10μM	4.8	1.4
Ex.11	tangeretin	100μM	12.2	3.5
Com.Ex.1	none (control)	-	3.5	1.0
Com.Ex.2	dibutyl cyclic AMP	100μM	11.6	3.3
Com.Ex.3	Isobutylmethyl-xanthine	100μM	11.8	3.4

[1)] Relative value is the value obtained by dividing "the percentage of the cells with extended neurites" by the control value (Comparative Example 1).

[0073]

As shown in Table 3, in comparison with the control of Comparative Example 1, all of the test materials A to G used in Examples 1 to 11 have excellent neurite extending effect to cells. According to the results of Examples 1 to 11, the higher concentration the test materials that are added to the cells have, the greater the neurite extending effect is. From this regard, it is evident that all of the test materials A to G used in Examples 1 to 11 are useful as neurite extending agents.

[0074]

<Example 12: production of food products>

Using the test material A (an extract of Seihi) obtained in Example 1, food products having the following composition were prepared.

[0075]

[Table 4]

Component	Weight (kg)
extract of Seihi	2.0
soybean saponin	2.0
black vinegar extract	2.0
apple fiber	2.0

lecithin	1.0
fructo-oligosaccharide	2.0
fructose	1.0
powdered vinegar	0.1
cyclodextrin	1.0
honey	1.0
bone dust	1.0
dextrin	4.9

[0076]

The components were mixed in granulator, and granulated with spraying water. Then, granules obtained were dried at blowing temperature of 80°C.

[0077]

<Example 13: production of hard gelatin capsules>

Using the test material C (an extract of Hirami-lemon) obtained in Example 3, hard gelatin capsules having the following were prepared.

[0078]

[Table 5]

Component	Quantity (mg/capsule)
extract of Hirami-lemon	250
starch	100
cellulose	100
magnesium stearate	10
total	460 mg

[0079]

<Example 14: production of tablets>

Using the test material D (nobiletin) described in Example 7, tablets having the following composition were prepared.

[0080]

[Table 6]

Component	Quantity (mg/tablet)
nobiletin	250
cellulose	400
silicon dioxide	10
magnesium stearate	5
total	665 mg

[0081]

[Effect of the Invention]

According to the present invention, an excellent neurite extending effect on cells with highly safe can be provided. In particular, to contain nobiletin or tangeretin that is a polyalkoxyflavonoid as an active ingredient is useful. The neurite extending agent of the present invention can be used as a medicament, a quasi-drug or a food product, and to prevent and/or treat neurodegeneration diseases such as Alzheimer's dementia and encephalic ischemia.

[Name of Document] ABSTRACT

[Abstract of the Disclosure]

[Problems to be Solved] To provide a neurite extending agent having a neurite extending effect without any sideeffects.

[Means for Solving the Problems] It was found that a polyalkoxy-flavonoid having a specific chemical structure, in particular, nobiletin or tangeretin, has a neurite extending effect. Furthermore, it was found that an extract from plant containing these compound has a neurite extending effect.

[Selected Figure] None